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The control of herpes simplex virus type-1 late gene transcription: a 'TATA-box'/cap site region is sufficient for fully efficient regulated activity

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ABSTRACT

The transcriptional programme of herpes simplex virus type 1 (HSV-1) is organised into three principle phases; immediate-early (IE), early (E) and late. The appearance of IE gene products provides the switch for E transcription. Abundant expression of late genes requires viral DNA replication. There is some overlap between E and late genes according to their degree of dependence on DNA replication. The pattern of expression of gene US11 is regulated with 'true-late' kinetics (Johnson et al., 1986) . In a transient assay system, regulation of a plasmid-borne US11 promoter mimics its viral counterpart, and has a similar dependence on DNA replication for abundant expression. Using plasmids which contain a functional HSV-1 origin of replication (ORI_S), we have identified the sequence requirements for the expression of late genes. All DNA sequence elements necessary for fully efficient regulated expression of US11 lie within 31 bp of the RNA cap sites; therefore it appears that a late gene promoter consists only of a proximal 'TATA-box' and cap-site region. We tested this hypothesis by removing the distal upstream region of the gD promoter (which is required for its normal regulation as an early promoter) and linking this truncated promoter to ORI_S. This resulted in the conversion of gD promoter regulation to late gene kinetics during virus superinfection. The implications of these results for the mechanisms of HSV gene regulation are discussed.

INTRODUCTION

Organization of the promoter sequences of many eukaryotic genes frequently includes; (i) a proximal element bearing 'TATA-box' homology; (ii) distal elements, typified by Sp1 binding sites and 'CAAT-box' homologies; and (iii) non-essential regulatory elements, which may contain enhancer activity and/or be required for response to regulatory trans-acting factors; these elements are sometimes located far-upstream (reviewed in 1,2,3). The systematic and detailed study of the transcriptional regulation of herpes simplex

virus type 1 (HSV-1) has provided many insights concerning eukaryotic gene control: Productive infection of herpes simplex virus type 1 (HSV-1) involves the coordinate temporal control of the viral transcriptional programme (reviewed in 4,5). The immediate-early (IE or α) genes, which encode the first viral transcripts to appear during infection and are defined as those genes that can be transcribed in the absence of de novo protein synthesis (6,7). A further feature distinguishes IE genes: their transcription is stimulated by a component of the virus particle, Vmw65 (8,9); this requires the presence of a far-upstream region including the consensus sequence, TAATGARATTC (10,11,12,13). These criteria clearly and unambiguosly define an IE gene. Not so easily defined are all the remaining of HSV genes, which have been broadly classified as early or late according to their expression kinetics. The early (E or β) genes require prior synthesis of IE gene products for their expression (7,14,15,16), whilst late genes additionally require viral DNA synthesis for their maximal expression. However, the dividing line between "early" and "late" genes is not easily drawn since some genes, such as glycoprotein D (gD), are detected very early in infection but require DNA synthesis for their maximal expression, although they are expressed at moderately high levels in its absence (17,18). Descriptions of these genes include leaky-late, γ and γ_1 genes (4,5). "True late" (γ or γ_2) gene expression is severely curtailed under conditions of DNA synthesis inhibition (19,20,21), but is nevertheless detectable by sensitive assays (18,22). Fully efficient regulated activity of characterised early and delayed-early genes requires proximal 'TATA' homologies and distal sequences (23,24,25,26).

For improved understanding of late gene regulation it is necessary to investigate the molecular mechanisms involved. We have studied gene US11, which encodes a polypeptide of apparent mol. wt. 21K (27), and is regulated with the kinetics and dependence on DNA replication characteristic of a "true late" gene (18). In a transient assay system using a plasmid containing an HSV origin of replication (ORI^+) and a US11

promoter/rabbit β-globin gene fusion, the US11 promoter was expressed with similar kinetics to its viral counterpart after infection of transfected cells with HSV-1 (28). Expression of US11 from ORI⁻ plasmids was detectable but severely curtailed, indicating that DNA replication, although not an absolute requirement for activation of the plasmid-borne US11 promoter, is essential for achieving abundant expression (28). One explanation for these results would be that late genes are activated similarly to early genes, but have relatively weak promoters which are more dependent on high copy number for abundant expression. It is alternatively possible that DNA replication in some way increases late gene expression by alteration of the state of the template. However, it is not clear why late gene products should become more abundant than early gene products after DNA replication.

Early gene promoters respond to the trans-acting factors Vmw175 and Vmw110, the products of IE genes 3 and 1 (15,16,29, 30). Because Vmw175 and Vmw110 are required for the expression of early gene products essential for replication, it is not clear whether they are also directly required for activation of true late gene expression. A third HSV IE product, Vmw63 is also important for full expression of some late genes (31). The trans-acting factors involved in late gene activation have not been well defined. A 'TATA-box' homology is located proximal to the US11 RNA cap sites; however, we noticed that distal sequence elements required for early gene activation (e.g. 'CAAT' box, GC- and GA-rich motifs: 23,24,25,32) did not appear to be present in the US11 upstream region (33).

In this paper we describe the sequence requirements for properly regulated expression of US11. A sequence of only 31 nucleotides 5' to the US11 RNA start site is sufficient for abundant expression from a replicating (ORI⁺) template. There are no sequences 5' to the US11 'TATA-box' which resemble elements required for early gene activation, and it seems that no others are substituted. We predicted that if a proximal 'TATA-box' is sufficient for late gene expression then it should be possible to convert an early HSV gene into a 'late'

by removal of sequences 5' to its 'TATA-box'. Expression from the early gD promoter, deleted to -33, was low at early times and abundant at late times on ORI$^+$ plasmids. In both cases transcription through the proximal 'TATA- box' promoters from an upstream IE promoter had a positive effect on 'late' expression. These results demonstrate a clear difference between the promoter structure of late genes and those of earlier classes, and raise important questions concerning the differential mechanisms underlying early and late gene regulation.

MATERIALS AND METHODS

1. Cells and virus

HeLa cells used for transfections were grown in Dulbecco's modified Eagles medium supplemented with 2.5% calf serum and 2.5% foetal calf serum (Flow Laboratories). HSV-1 strain 17 syn$^+$ was used for infection of transfected cells (34).

2. Plasmids

Plasmids pPJ2, pPJX5, pPJS5, pRED4, pRED5, pβ(244+)β, pERD130/33 (pERD7.122) and pERD130/83 (pERD7.119) have been described (25,28,35). The construction of the pPJO series of deletions into the US11 promoter, and pOR/RO plasmids is outlined in the Results section.

3. Calcium phosphate transfection, infection of HeLa cells, RNA isolation and S1 mapping analysis

Subconfluent layers of HeLa cells on 90mm plates were transfected as described (25) using 10ug of the test plasmid and 10ug of internal control plasmid, pRED5 (28) or pβ(244+)β, (35). Viral infections were performed 24h. after transfection and RNA prepared at early (4 hr) or late (16 hr) times after infection. The methods used for isolation and S1 mapping of RNA are as described for mapping gD- initiated transcripts (35). The probe used for mapping US11-hybrid transcripts was the single stranded DNA fragment from pPJ2 shown in Figure 1B, ^{32}P end-labelled at the BstNI site, position +136 in the globin gene. This probe detects RNA transcripts initiated from the capsites of pPJ2 and pPJ2-derivatives. In addition, it detects RNA originating from the the internal control

plasmid pRED5, resulting in S1 resistant bands which map to the break in homology at the gD/globin junction. Suitably exposed autoradiographs were analysed by densitometry, as described (18,28).

RESULTS

Construction of US11 promoter deletion mutants

We have previously described the construction and structure of plasmid pPJ2, which contains the HSV-1 US11

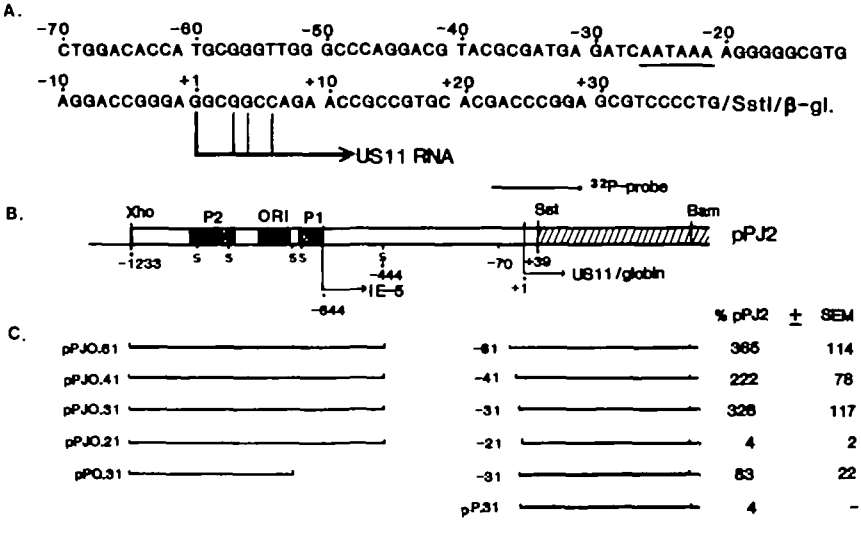

Fig.1 Detailed structure and sequence of the US11/β-globin hybrid gene promoter region. A. DNA sequence of the US11 promoter region, -70 to +39, in pPJ2. The positions of the RNA starts and the consensus 'TATA-box' are indicated. B. Structure of pPJ2. HSV-1 US11 DNA (-1233 to +39) is joined to rabbit β-globin DNA at a SstI linker. Indicated upstream from the US11 transcription start (+1): IE gene 5 transcription start site and promoter (P1); TR_S origin of replication (ORI); IE gene 5 far-upstream activating sequence (P2). The positions of relevant restriction sites are shown, including SmaI sites (s). The probe used for detecting US11 initiated and pRED5 RNA (which share homologous globin sequences), ^{32}P- labelled at a BstN1 site, +136 in the globin gene, is shown above pPJ2. C. Structures and activities of mutant test plasmids. Gaps represent deleted sequences, whose 3' end-points align to the indicated SmaI sites, and are joined by a SalI linker to 5' deletions (-21 to -61) of the US11 promoter. The 3' end-point of pP31 is the SalI site of pBR322. The mean relative transcriptional activities (+/- standard error of the mean; SEM) of the transfected mutant constructs after 16 hrs viral infection, are shown on the right (pPJ2 = 100%).

promoter linked to the rabbit β-globin gene in a pBR322 based vector (28). This plasmid also contains the HSV-1 IE gene 5 promoter and its far-upstream regulatory sequences, and the TR_S/IR_S origin of DNA replication (ORI_S) in their normal locations 5' to the US11 gene (12,36). We have reported that deletion of ORI_S from pPJ2 leads to grossly diminished transcription of the US11/globin gene during HSV-1 infection of transfected cells (28). Therefore, in order to define the DNA sequences that comprise the US11 promoter, we have constructed deletion mutants in plasmids which contain ORI_S.

The structural features of pPJ2 that are relevant to this study are shown in Figure 1B. Deletions extending towards the US11 cap-sites were isolated after Bal31 nuclease digestion of XhoI-cut pPJX5, a derivative of pPJ2 containing a XhoI site at -444 (28). In all cases, a SalI linker was inserted at the limit of the deletion. Plasmid pPJS5 is a derivative of pPJ2 which contains the promoter and regulatory sequences of IE gene 5 and ORI_S, and has a SalI linker at position -444 (28). Thus fusion of the SalI site of a deletion plasmid to the SalI site of pPJS5 created the pPJO.n series of plasmids (Figure 1C), where 'n' refers to the number of nucleotides 5' to the US11 start site that remain before the SalI linker.

Transcriptional activation of the pPJO series

Plasmids were transfected into HeLa cells with an internal control plasmid, pRED5, to allow standardization of the transfection procedure. The cells were washed 24 hours after transfection, infected with HSV-1, and RNA isolated 16 hr after infection. Correctly initiated US11 hybrid transcipts were detected by S1 mapping, using a single stranded DNA probe, 5' end-labelled with ^{32}P as shown in Figure 1B. pRED5 contains the gD promoter linked to the rabbit β-globin gene under control of the SV40 enhancer. Transcripts from pRED5 were detected by the same probe and give rise to bands corresponding to the break in homology at the HSV/globin junction (Figure 2). Autoradiographs of S1 gels were quantitated by densitometry. The level of correctly initiated US11 RNA from the promoter deletion series of plasmids was compared to the level from pPJ2, after normalization of the

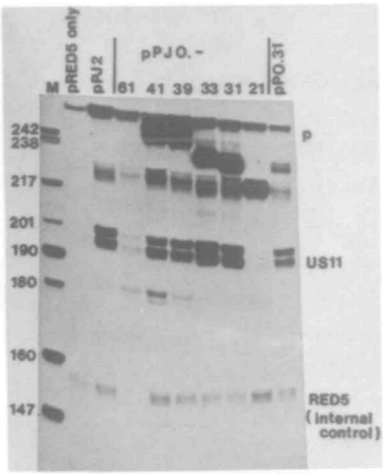

Fig.2 S1 analysis of transcriptional activation of mutant US11 promoter constructs after superinfection of transfected cells with HSV-1 for 16 hrs. RNAs produced from the US11 hybrid gene and pRED5 are marked. Transfections included 10ug of test plasmid and/or 10ug pRED5 control plasmid, as indicated. The band marked 'p' corresponds to full length probe. Size standards (M) are HpaII- cut pBR322 DNA. The ratio of US11 initiated RNA compared to pRED5 RNA was determined by densitometry, and the averaged results from multiple experiments are given in Fig.1C. The poor detection of both US11 and pRED5 RNA in the pPJO.61 track demonstrates the importance of the internal control.

individual transfection efficiencies (determined by the level of detected RNA corresponding to pRED5).

Preliminary experiments indicated that US11 expression from pPJO plasmids with deletions extending to -70 was not impaired. We therefore concentrated our analysis on constructs with deletion end-points downstream from -70. The results of a typical transfection experiment are shown in Figure 2, and a summary of data derived from multiple transfections using pPJO.61,41,31 and 21 is given in Figure 1C. Deletion of sequences upstream from the US11 cap sites had no significant effect on inducible promoter activity until the deletion end-point had passed -31. The observation that sequences 3' of -32 are sufficient for abundant US11 transcription was striking. Most of the 'TATA-box' (see Figure 1A) is lost in pPJO.21, so the corresponding 25-fold decrease in the level of correctly initiated transcripts from this construct was not suprising. Higher levels of expression were reproducibly observered from the pPJO series of plasmids (deletion end-points -31 or upstream) compared to pPJ2 (Figure 1C). If DNA replication and through transcription from the IE gene 5 promoter potentiate the US11 promoter by opening up

the template structure (28), then the increased transciption from pPJO.61, pPJO.41 and pPJO.31 may be because the initiation of both replication and IE gene 5 transcription is closer to the US11 promoter in these plasmids than in pPJ2. However, the possibility of sequences required for a negative effect on US11 transcription, contained within the region -444 to -61, cannot be ruled out.

These results suggest that the US11 promoter does not have an essential distal promoter region analagous to those of the HSV-1 tk and gD promoters (24,25): the 'TATA-box' and capsite region are necessary and sufficient to allow fully efficient regulated expression. However, it could be argued that the US11 promoter does have a functional distal promoter region, but that sequences 5' to the SalI linker at position -444 are able to substitute for it in the pPJO deletions. We consider this is an unlikely explanation for three reasons. Firstly, the exact distance between a distal region and its associated 'TATA-box' is known to be crucial for full activity of the tk and SV40 early promoters (37,38,39); whereas the distance from the US11 'TATA-box' and the sequences 5' to the SalI linker varies by 30 bp between pPJO.61 and pPJO.31, with no significant change in promoter activity. Secondly, deletion of sequences 5' of the SalI linker, up to and including the IE gene 5 promoter (in plasmid pPO.31, Figure 1C) resulted in a promoter only slightly less efficient than that in pPJ2. Thirdly, inversion of the SalI fragment in pPO.31, containing part of the bacterial tetracycline resistance gene and HSV-ORI_S, created pPO'.31, which also gave similar levels of US11 promoter activity compared to pPO.31 and pPJ2 (data not shown). The sequences adjacent to the US11 'TATA-box' in pPJO.31, pPO.31, pPO'.31 and pPJ2 are entirely different which implies that they are not providing a distal upstream promoter region.

Effects of replication and transcription through the -31 US11 promoter

Previous studies have indicated that transcription through the US11 promoter has a positive effect on US11 transcription (28). The effects of an absence of IE gene 5 transcription on

the activity of the minimal (-31) US11 promoter are illustrated by plasmid pPO.31. US11 transcription from pPO.31 was reduced only slightly compared to pPJ2, but around 4-fold compared to pPJO.31 (Figures 1C and 2). Inducibility of the US11 promoter in the absence of IE 5 promoter indicates that IE gene 5 does not provide surrogate promoter functions for US11, but does support the conclusion that transcription through the US11 promoter increases its expression.

In the absence of ORI_S or other HSV sequences upstream from -31, the US11 promoter was poorly expressed (pP.31: see Figure 1C). The level of expression from pP.31 was similar to an undeleted US11 promoter in an ORI^- environment (28).

Does a 'TATA-box' constitute a functional late promoter?

The experiments described in the preceeding sections show that HSV-1 sequences between -31 and +39 are sufficient for efficient expression of US11. This region includes a consensus 'TATA-box' homology beginning at position -26, as indicated in Figure 1A. If a 'TATA-box' alone is sufficient for late gene expression in an ORI^+ environment, it should be possible to induce 'late' expression from the 'TATA-box' of other promoters, which do not otherwise share homology with US11. We decided to test this hypothesis using a deletion mutant of the HSV-1 gD promoter. The necessary proximal and distal promoter elements for normal early kinetic gD promoter expression lie within 83 bp of the gD mRNA capsites (35). A 'TATA-box' is located at position -25 to -20 (Figure 3A), and has been shown to be essential for accurate initiation of gD transcription (25). Deletion of the distal promoter sequences leads to a >25-fold decrease in gD promoter activity in the absence of DNA replication (35).

Construction of a 'late' gD promoter

We decided to examine the transcriptional activity of a -33 deletion mutant of the gD promoter, which retains a 'TATA-box', in an ORI^+ environment. The IE gene 5 regulatory sequences and ORI_S from pPJS5 (Figure 3B and described in ref.28) were placed upstream from a -33 deletion-mutant of gD (pRED.122: ref.35), to give pOR33 (Figure 3C). Induction of pOR33 was compared with pERD.130/33, which has gD sequences

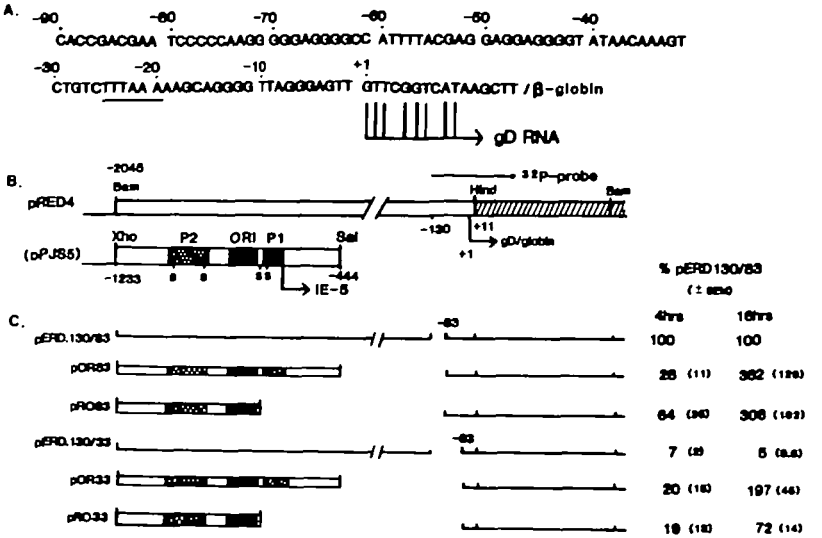

Fig.3 Detailed structure and sequence of the gD/β-globin hybrid promoter region. A. DNA sequence of the gD/globin promoter, from -90 to +11, in pRED4. The positions of the RNA starts and the consensus 'TATA-box' are indicated. B. Structure of pRED4. HSV-1 gD DNA (-2045 to +11) is joined to rabbit β-globin DNA at the HindIII site beginning at +11. The probe derived from pRED4 for detecting gD- and globin-initiated RNA (labelled at +136 of β-globin) is shown above. The structure of the HSV-1 origin containing fragment from pPJS5 (a pPJ2 derivative) is shown below pRED4. C. Structures and activities of the mutant test plasmids. Deletions into the gD promoter, 5' delineated by a XhoI linker, are joined to: (i) -2045 to -130 gD upstream DNA (3' XhoI) - pERD.130/n; (ii) -1233 to -444 US11 upstream DNA (3' SalI) - pORn; or (iii) -1233 to -714 US11 upstream DNA (3' SalI) - pROn, where 'n' is the -83 or -33 gD promoter deletion end-point. The mean relative transcriptional activities (+/- SEM) after 4 or 16 hrs viral superinfection are shown on the right (pERD.130/83 = 100%). Note that the results at the two time points are not directly comparable since between 4 and 16 hrs superinfection, the accumulation of plasmid-derived gD transcripts in HeLa cells is reduced by around 20%.

-2045 to -130 incorporated upstream from the -33 deletion end-point (Figure 3C). pERD130/33 provides a negative control for induction of the -33 deleted gD promoter in an ORI$^-$ environment. To eliminate possible surrogate promoter effects by IE gene 5 in pOR33, an ORI$_S$-containing fragment from pPJS3, which lacks the IE gene 5 promoter, was placed upstream from the -33 deletion to create pRO33 (Figure 3C). For

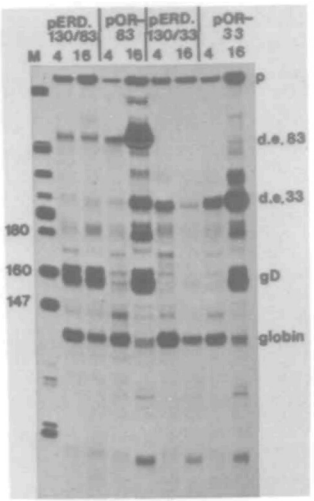

Fig.4 S1 analysis of transcriptional activation of mutant gD promoter test plasmids after 4 or 16 hrs viral activation. Transcripts initiated from gD test plasmids and the β-globin internal control plasmid, p (244+) , are marked. Full length probe (p) and bands corresponding to the break in homology at the -83 and -33 deletion end-points (d.e.), are indicated.

comparison with the -33 promoter, analagous constructs were made using a -83 promoter deletion (pERD130/83, pOR83 and pRO83), which retain all the cis- DNA sequence elements for properly regulated expression of gD (25).

Transcriptional activation a gD 'TATA-promoter'

Transcriptional activation of the gD promoter constructs shown in Figure 3C was examined in transfection assays at early (4 hr) or late (16 hr) times after infection with HSV-1. The relative promoter activities were determined by quantitative S1 mapping using the ^{32}p 5'-end labelled single stranded DNA probe indicated in Figure 3B. Standardization of the transfection procedure was by reference to the level of globin transcripts from a co-transfected internal control plasmid pβ(244+)β (35), indicated in Figure 4. The activities of the various gD promoter constructs were compared to and expressed as percentages of the level from pERD130/83. Since both the level of accumulated globin (Figure 4, and data not shown) and gD transcripts decrease at late times (18), the activities determined at different time-points shown in Figure 3C are not directly comparable.

In an ORI$^-$ environment, induction of the -33 gD promoter in pERD130/33, is poor at both early and late times (Figure 3C and 4), consistent with previous findings (25,35). The

addition of ORI_S to the -33 gD promoter in pOR33 and pRO33 produced a very dramatic increase in gD initiated transcripts at late times (Figures 3C and 4). This result demonstrates that a 'TATA-box' can function as a late promoter in an ORI^+ environment. Deletion of the IE gene 5 promoter in pRO33 reduced gD promoter activity at late times compared to pOR33: Transcription through the gD 'TATA-promoter' thus has a similar effect as seen with the US11 promoter (Figure 1C and 3C).

Some less predictable effects were observed with the introduction of ORI_S to plasmids containing a complete or deleted gD promoter. At early times, pOR33 and pRO33 were usually moderately active (Figure 3C). This early activity probably occurred in assays where DNA replication had commenced before cells were harvested. We also noticed that at early times the presence of ORI_S reduced gD expression from the -83 promoter (pRO83) and this reduction was more marked in the presence of the IE gene 5 promoter (pOR83: Figure 3C and 4). It is interesting that through transcription and replication appear to have the converse effect compared to that on US11, they down regulate a functional early promoter at early times (pOR83 and pRO83; Figure 3C). This may be similar to the repression of transcription from the SV40 early promoter by viral DNA replication reported by Lewis and Manley (40). In contrast, at late times ORI_S is responsible for increased activity of the -83 promoter (Figure 3C). This observation is consistent with studies of viral gD RNA accumulation, which is reduced in the presence of DNA replication inhibitors (18).

DISCUSSION

Features that distingish an HSV late promoter and account for its characteristic late induction kinetics have been identified. The US11 gene of HSV-1 is regulated with 'true-late' kinetics (18), and a plasmid-borne US11 promoter can mimick these kinetics in a transient assay system (28). DNA replication is not required for activation of late gene expression, but is very important for achieving abundant

expression (18,22,28,41). For this reason we investigated the function of US11 promoter deletions by locating them on plasmids containing an HSV-1 origin of replication (ORI_S), which enables replication of transfected plasmid DNA in the presence of infecting HSV (28,36). We have shown that the DNA sequence elements required for fully efficient regulated expression of US11 lie within 31 bp of the RNA cap sites.

Visual comparison of the DNA sequence upstream from the US11 cap sites with the well characterised tk and gD promoters yielded no obvious homology, with the exception of a proximal consensus 'TATA-box' region (24,25). However, the sequence tract between the 'TATA-box' and cap sites of both the US11 and gD promoters is notably purine rich (80% and 73% respectively). The degree of purine richness in the same region of other HSV promoters is not always so high; e.g. tk- 55%; VP5- 50%; gC- 70% (5); and the significance (if any) of a purine-rich tract in the region, approximately -20 to -1, is not known. Thus it seemed possible that an HSV late gene may be characterised by the presence of a 'TATA-box' in the absence of distal regulatory sequences upstream. We predicted that the early gD promoter might be regulated with late kinetics after (a) removal of sequences upstream from its 'TATA-box' and (b) linkage to the ORI_S origin of DNA replication. This prediction was confirmed with constructs pOR33 and pRO33, both of which contain an upstream to -33 deletion of the gD promoter in an ORI^+ environment that is inducible at late times after infection with HSV-1 (Figure 3C). These results demonstrate that there is a clear difference in sequence requirements for proper regulation of a model "early" and a model "late" promoter.

Previous studies have shown that transcription through the US11 promoter starting from the upstream IE gene 5 promoter plays a positive role in achieving abundant US11 transcription (28). The results shown here suggest that transcription through either the minimal US11, or the 'converted late' gD promoter increases standard late promoter activity. In the pPJO series of constructs and pOR33 (Figures 1C and 3C) the IE gene 5 promoter (in the ORI_S-containing fragment) was placed

immediately upstream from the US11 and gD deletion end-points. Conceivably this might have enabled the IE gene 5 regulatory region to provide surrogate promoter functions on US11 and gD. This possibility was dismissed by constructs pPO.31 and pRO33, in which the IE gene 5 promoter had been deleted. Despite the complete change of sequences upstream of their 'TATA box' regions, both promoter constructs exhibited substantial activities at late times (Figure 1C and 3C). Furthermore, inversion of an ORI_S-vector fragment in pPO.31 and pRO33 (which places pBR322 sequences immediately upstream of the US11 and gD 'TATA box' regions) did not alter the level of induced US11 or gD expression (data not shown). This indicated that the position or nature of the DNA sequences upstream of the deleted promoters made no essential contribution to their inducible expression at late times after infection.

If US11 is typical of true-late HSV promoters [in fact, it has recently been shown by Homa et al., (42) that another HSV-1 true late gene, gC, has similar sequence requirements (-34 to +124) for proper regulated expression], then the relationship between the three temporal classes of HSV promoters may be summarised as shown in Figure 5. This illustrates that as infection proceeds and after DNA replication occurs, fewer regulatory signals are required for efficient transcription from a promoter. In the case of US11, an active origin of DNA replication appears to substitute for a distal ("upstream") promoter region during the later stages of the lytic cycle. Thus late gene promoters may not require certain cellular transcription factors, such as Spl, that IE and early genes have been shown to require (32,43); these cellullar factors may become scarce or diluted out at the later stages of the lytic cycle, due to HSV mediated shutoff of host mRNA and polypeptide synthesis (44,45) and build up of HSV template DNA. It is interesting to speculate that the relaxation in the requirements for cis-acting DNA sequences for transcription initiation may lead to an increase in apparently randomly initiated RNA molecules, which may account for at least a proportion of the increased "symmetric"

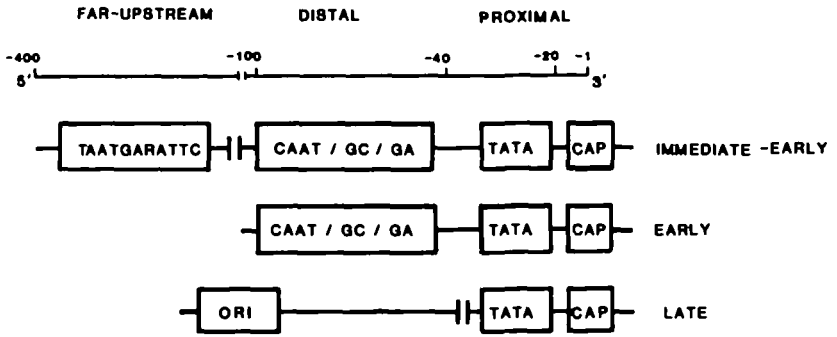

Fig.5 Regulatory signals required in cis for efficient temporal expression of HSV-1 genes. The far-upstream element of IE genes, required for activation by the virion component, Vmw65, includes the consensus sequence, TAATGARATTC, in addition to flanking modulatory sequences. The distal promoter elements of IE and E genes contain one or more copies of at least one of the following: (i) GC-rich motifs, (ii) GA-rich motifs, (iii) a 'CAAT-box'. Late promoters, which contain only a 'TATA-box' - capsite region (CAP), require an active origin of replication (ORI) in cis for their efficient expression.

transcription (that occuring on both DNA strands of a gene) observed at late times associated with DNA replication (41,46).

Efficient activation of a number of early genes has been shown to be mediated by IE gene 1 and 3 products (Vmw175 and 110: refs 15,16,29,47). Many early promoters contain a consensus 'CAAT-box', and GC- or GA-rich motifs in their distal promoter regions, in addition to a proximal 'TATA-box', which have been shown to be required for their trans-activation by Vmw175 and Vmw110 (15,24,26,32). A study of the gD promoter did not identify any specific sequences required for viral trans-activation that were not also needed for cis-activation (25), which suggested that IE gene products activate E gene transcription by either (i) binding sequences recognised by cellular transcription factors, or (ii) altering the activity of cellular transcription factors. Recent studies have suggested that late promoters can also be activated, in the absence of DNA replication, by IE gene products. Activation of a late promoter, L42 (21), by co-transfection with IE genes has been detected using

sensitive CAT and tk assays, in transient assay systems (47,48). Preliminary results using a minimal (-31) US11 promoter/CAT fusion gene have shown that CAT activity can be induced by Vmw175 and Vmw110, and we have recently been able to detect US11 promoter activity in co-transfection experiments by S1 mapping (unpublished data). If Vmw175 and Vmw110 activate late gene expression, then the sequences required for their action (directly or indirectly) may be within the 'TATA-box'- cap-site region, since this is all that constitutes a late promoter.

Finally, we note that although transcription of some early genes is increased by DNA replication (perhaps simply due to an increase in copy number), their expression is decreased at late times (7,18). The difference between late and early promoters at the sequence level suggests a mechanism to turn down early transcription without affecting late gene expression: Factors that bind to distal upstream regions to affect transcription may be inhibited, or their function altered causing inhibition of early promoter activity. Thus the mechanisms for both activation (in conjunction with IE gene products) and repression of early genes may be mediated by cellular transcription factors without recourse to virus-specific sequences.

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REFERENCES

1. Breathnach,R. and Chambon,P. (1981) Ann. Rev. Biochem., 50, 345-383.
2. Serfling,E., Jasin,M. and Schaffner,W. (1985) Trends Genet. 1, 224-230.
3. Kadonaga,J.T., Jones,K.A. and Tijan,R. (1986) Trends Biol. Sci.,11, 20-23.
4. Roizman,B. and Batterson,W. (1985) In Fields,B.N. (ed.) Virology, Raven Press, New York, Ch.25 pp497-526.
5. Wagner,E.K. (1985) In Roizman,B. (ed.) The Herpesviruses Vol. 3, Plenum Press, Ch.2 pp45-104.
6. Kozak,M. and Roizman,B. (1974) Proc. Natl. Acad. Sci. USA, 71, 4322-4326.
7. Honess,R.W. and Roizman,B. (1974) J.Virol., 14, 8-19.
8. Post,L.E., Mackem,S. and Roizman,B. (1981) Cell, 24, 555-565.

9. Campbell,M.E.M., Palfreyman,J.W. and Preston,C.M. (1984) J. Mol.Biol., 180, 1-19.
10. Mackem,S. and Roizman,B. (1982) J.Virol., 44, 939-949.
11. Whitton,J.L., Rixon,F.J., Easton,A.J. and Clements,J.B. (1983) Nucleic Acids Res., 11, 6271-6287.
12. Preston,C.M., Cordingley,M.G. and Stow,N.D. (1984) J.Virol., 50, 708-716.
13. Bzik,D.J. and Preston,C.M. (1986) Nucleic Acids Res., 14, 929-943.
14. Preston,C.M. (1979) J.Virol., 29, 275-284.
15. Everett,R.D. (1984a) EMBO J., 3, 3135-3141.
16. O'Hare,P. and Hayward,G.S. (1985) J.Virol., 53, 751-760.
17. Gibson,M.G. and Spear,P.G. (1983) J.Virol., 48, 396-404.
18. Johnson,P.A., MacLean,C., Marsden,H.S., Dalziel,R.G. and Everett,R.D. (1986) J. Gen. Virol., 67, 871-883.
19. Jones,P.C. and Roizman,B. (1979) J.Virol., 31, 299-314.
20. Holland,L.E., Anderson,K.P., Shipman,C. and Wagner,E.K. (1980) Virology, 101, 10-24.
21. Hall,L.M., Draper,K.G., Frink,R.J., Costa,R.H. and Wagner,E.K. (1982) J.Virol., 43, 594-607.
22. Godowski,P.J. and Knipe,D.M. (1985) J.Virol., 55, 357-365.
23. McKnight,S.L. and Kingsbury,R. (1982) Science, 217, 316-324.
24. Eisenberg,S.P., Coen,D.M. and McKnight,S.L. (1985) Mol. Cell. Biol., 6, 1940-1947.
25. Everett,R.D. (1984b) Nucleic Acids Res., 12, 3037-3056.
26. Costa,R.H., Draper,K.G., Devi-Rao,G., Thompson,R.L. and Wagner,E.K. (1985) J. Virol. 56, 19-30.
27. Rixon,F.J. and McGeoch,D.J. (1984) Nucleic Acids Res., 12, 2473-2487.
28. Johnson,P.A. and Everett,R.D. (1986) Nucleic Acids Res., 14,3609-3625.
29. Gelman,I.H. and Silverstein,S. (1985) Proc. Natl. Acad. Sci. USA, 82, 5265-5269.
30. Quinlan,M.P., and Knipe,D.M. (1985) Mol. Cell. Biol., 5, 957-963.
31. Sacks,W.R., Greene,C.C., Aschman,D.P. and Schaffer,P.A. (1985) J.Virol., 55, 796-805.
32. Jones,K.A., Yamamoto,K.R. and Tijan,R. (1985) Cell, 42, 559-572.
33. McGeoch,D.J., Dolan,A., Donald,S. and Rixon,F.J. (1985) J. Mol.Biol., 181, 1-13.
34. Brown,S.M., Ritchie,D.A. and Subak-Sharpe,J.H. (1973) J. Gen. Virol.,18, 329-346.
35. Everett,R.D. (1984b) Nucleic Acids Res., 12, 3037-3056.
36. Stow,N.D. and McMonagle,E.C. (1983) Virology, 130, 427-438.
37. Moreau,P., Hen,R., Wasylyk,B., Everett,R.D., Gaub,M.P. and Chambon,P. (1984) Nucleic Acids Res. 9, 6047-6067.
38. McKnight,S.L., Kingsbury,R.C., Spence,A. and Smith,M. (1984) Cell 37, 253-262.
39. Takahashi,K., Vigneron,M., Matthes,H., Wildeman,A., Zenke,M. and Chambon,P. (1986) Nature 319, 121-126.
40. Lewis,E.D. and Manley,J.L. (1985) Nature, 317, 172-175.
41. Godowski,P.J. and Knipe,D.M. (1986) Proc. Natl. Acad. Sci. USA, 83, 256-260.

42. Homa,F.L., Otal,T.M.,Glorioso,J.C. and Levine,M. (1986) Mol. Cell.Biol. (*in press*).
43. Jones,K.A. and Tjian,R. (1985) Nature 317, 179-182.
44. Fenwick,M., Morse,L.S. and Roizman,B. (1979) J.Virol., 29, 825-827.
45. Read,G.S. and Frenkel,N. (1983) J.Virol., 46, 498-512.
46. Jaquemont,B. and Roizman,B. (1975) J.Virol., 15, 707-713.
47. DeLuca,N.A. and Schaffer,P.A. (1985) Mol. Cell. Biol., 5, 1997-2008.
48. Mavromara-Nazos,P., Silver,S., Hubenthal-Voss,J., McKnight,J.L.C. and Roizman,R. (1986) Virology 149, 152-164.